FINGER PRINT OF LUMBAR PUNCTURE CEREBROSPINAL CATECHOLAMINES, TRYPTOPHAN AND THEIR METABOLITES IN PATIENTS WITH NEUROLOGIC DISORDERS

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Abstract

The levels of catecholamine, tryptophan and their metabolites in lumbar cerebrospinal fluid (CSF) are reported in various neurologic patients such as Parkinson's disease (PD), cerebrovascular disorders (CVD), multiple sclerosis (MS), tuberculous meningitis (TBM) and aseptic meningitis (AS). These results are statistically compared with healthy subjects, and results are discussed to utilize the information to differentiate between these neurologic disorders. Besides, the information collected can be utilized in understanding these neurologic disorders in developing drug therapy.

Key Words

Catecholamines, serotonin, cerebrospinal fluids, neurologic disorders

Introduction

The nervous system is an organ system that coordinates sensory information and generates appropriate behavioural responses. All cells of an organism have some ability to respond to external stimuli by changes in the cell membrane but nerve cells are specialized for this task. Neurons are specialized for short and long distance transmission throughout the body. The present knowledge suggests that the information which is transmitted through the CNS is both in the form of electrical and chemical signals. The electrical signals are nerve impulses that travel along the cell membrane and trigger the release of chemical signals (transmitters) in the synapse. The released transmitters initiate an electrical signal in the next neuron or other target cell.

Transmission of nerve impulses from one neuron to another or from a neuron to a peripheral effector cell such as muscle cells is a critical event in the nervous system. Its elucidation is essential not only for our understanding of normal neuronal function, but it is highly probable that changes in the chemical transmission process may underlie or at least are related to various disease processes in nervous system. The catecholamines dopamine (DA), noradrenaline (NA) and the indoleamine, 5-hydroxytryptamine (5-HT) act as important neurotransmitters in the brain. Abnormalities of biogenic amine metabolism in the central nervous system have been implicated in various psychiatric and neurological disorders. The estimation of monoamine and indoleamine are shown to be helpful for the diagnosis and interpretation of these disorders. In this study, CSF analysis of classical neurotransmitters such as catecholamines i.e. dopamine, noradrenaline and indoleamine tryptophan and their metabolites are reported in patients with various neurologic disorders such as Parkinson's disease (PD), cerebrovascular disorder (CVD), multiple sclerosis (MS), tuberculous meningitis (TBM) and aseptic meningitis and the comparison is made with healthy subjects.

Material and Methods

Patients and Healthy Subjects

All patients were recruited from the department of Neurology, Huddinge University Hospital, Sweden except

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patients with tuberculous meningitis who were recruited from the department of Neurology, the Aga Khan University Hospital, Karachi, Pakistan. CSF samples were obtained by lumbar puncture within 4-8 hours of admission. 20 Parkinson’s disease (PD) patients (7 females, with mean age 72±12 years), 16 patients (6 females, mean age 62±4 years) with ischemic stroke or cerebrovascular disorders (CVD), 20 patients (8 females, mean age, 54±6 years) with definite multiple sclerosis (MS), 18 patients (9 females, mean age, 46±5 years) with aseptic meningitis (AM) and 14 patients (5 females, mean age 51±4 years) with tuberculous meningitis (TBM) were included in this study. The diagnosis of PD was made according to established clinical criteria. None of these patients exhibited clinical indications of symptomatic parkinsonism or depression and all had shown clear response to L-DOPA. Among PD patients, 6 patients were included who did not receive L-DOPA and other DAergic drugs. For CVD patients, computerized tomography (CT scan) and/or MRI confirmed the evidence of stroke and its site. All the strokes were in territory around the middle cerebral artery. Ten of the patients had hemiplegia and 6 had hemiparesis at the time of admission. All the patients showed normal CSF cell count while CSF protein and IgG were significantly higher (Table 1). Multiple sclerosis was diagnosed according to Schumacher criteria. 9 of the MS patients had exacerbation, 5 had remission, while 6 had chronic progressive form of MS at the time of sampling. Exacerbation was defined as sudden appearance of new signs and symptoms or sudden worsening of previous signs and symptoms lasting more than 24 hours. All of these patients had oligoclonal bands in their CSF. Routine CSF examination showed mononuclear pleocytosis (>5x10^6/liter) in all patients. Since, there was no difference in the levels of CSF neuropeptides in exacerbation, remission and chronic progressive form, all MS patients are considered in one group.

Slightly raised CSF/serum albumin ratio reflecting low-grade blood-brain barrier damage was present in MS patients, and elevated IgG Index were observed. For meningitis patients, the diagnosis of the disease was based on standard diagnostic criteria. Besides, aseptic meningitis was diagnosed on the basis of clinical features, culture, sensitivity tests and laboratory investigations of CSF at the time of admission at the hospital. Subsequent examination of CSF was carried on the basis of clinical and therapeutic considerations. The diagnosis of tuberculous meningitis was established on clinical and CSF findings, positive cerebrospinal Gram stain and latex agglutination and positive presence of growth of pathogenic bacteria. CSF samples were also collected from 14 healthy individual (5 females, mean age 53±5 years) with complaints of muscular tension headache. These individual were considered as healthy subjects (HS) because CSF routine analysis as well as blood complete examination, liver function test, electrolytes, ESR were within normal limits. Their general physical examination, neurological observation and CT scan of head was also normal. The clinical data on the healthy subjects and patient groups are presented in Table 1. All values are expressed as mean ± Standard Error Mean (SEM).

Table 1: Clinical data on the healty subjects (HS), patients with Parkinson’s disease (PD), cerebrovascular disorders (CVD), multiple sclerosis (MS), aseptic (AM) and Tuberculous meningitis (TBM).

<table>
<thead>
<tr>
<th>Patients n Females Age (years)</th>
<th>CSF-albumin</th>
<th>CSF-IgG</th>
<th>IgG-index</th>
</tr>
</thead>
<tbody>
<tr>
<td>HS 14 5</td>
<td>53±5</td>
<td>218±17</td>
<td>34±4</td>
</tr>
<tr>
<td>PD 20 7</td>
<td>72±12</td>
<td>332±42*</td>
<td>51±7**</td>
</tr>
<tr>
<td>CVD 16 6</td>
<td>70±10</td>
<td>313±31*</td>
<td>46±6*</td>
</tr>
<tr>
<td>MS 20 8</td>
<td>54±6</td>
<td>188±14</td>
<td>65±7**</td>
</tr>
<tr>
<td>AM 18 9</td>
<td>46±5</td>
<td>412±53**</td>
<td>83±12***</td>
</tr>
<tr>
<td>TBM 14 5</td>
<td>51±4</td>
<td>442±61**</td>
<td>88±11***</td>
</tr>
</tbody>
</table>

*p<0.05, **p<0.01, ***p<0.001

Lumbar Puncture and Routine CSF Analysis

10-12ml cerebrospinal fluid (CSF) was collected from each patient and healthy subject in a sitting position at the L4-L5 levels. Blood samples were collected by venipuncture. The basic CSF analysis included: cell counting by phase-contrast microscopy, determination of CSF/serum albumin ratio and CSF/immunoglobulin G (IgG) index as well as isoelectric focusing for detection of oligoclonal IgG band. Serum and CSF albumin and IgG were determined using Hitachi 737 Automatic Analyzer (Naka Works, Hitachi Ltd., Tokyo, Japan).

Analysis of Catecholamines, Indolamines and their Metabolites

Both serum and CSF were kept at -70°C if not analyzed immediately. The CSF was prepared as previously described and the concentrations of DA, homovanillic acid (HVA), 3,4-dihydroxyphenylacetic acid (DOPAC), NA, 3-methoxy-4-hydroxyphenylglycol (MHPG), 5-hydroxytryptamine (5-HT) and 5-hydroxyindoleacetic acid.
(5-HIAA) were measured using previously described methods. All chemicals used were obtained from various commercial sources and were of Analytical- Reagent grade.

Analysis of Data

Data are presented as mean ±SEM. Differences in concentrations of catecholamines, tryptophan and their metabolites were analyzed with ANOVA and group comparison were made with t-test. A p-value less than 0.05 was considered significant.

Results

Fig. 1 shows the CSF levels of NA and its metabolite MHPG in healthy subjects (HS) and patients with various neurologic disorders.

As compared to HS, the level of NA is significantly increased from 106±5 pmol/l to 133±7 (p<0.05), 158±12 (p<0.01), 143±7 (p<0.05), 156±10 (p<0.01) and 144±8 (p<0.05) pmol/l in PD, CVD, MS, TBM and AM patients respectively. The level of MHPG was also significantly increased from 61±4 pmol/l to 75±4 (p<0.05), 78±3 (p<0.05), 86±7 (p<0.01) and 79±5 (p<0.05) in PD, MS, TBM and AM patients respectively, whereas unchanged level was found in CVD patients. Fig. 2 shows the CSF levels of DA and its metabolites HVA and DOPAC as compared to HS, DA level was decreased significantly from 53±4 pmol/l to 44±3 (p<0.05), 40±3 (p<0.05), 39±5 (p<0.05) in PD, MS and TBM patients, whereas unchanged levels were found in CVD and AM patients.

The level of HVA was decreased from 310±17 pmol/l to 265±11 (p<0.05), 254±14 (p<0.01) and 267±12 (p<0.05) pmol/l in MS, TBM and AM patients respectively and unchanged levels were found in both PD and CVD patients. The level of DOPAC was significantly decreased from 95±5 pmol/l to 83±6 (p<0.05), 66±8 (p<0.05), 67±10 (p<0.05), 78±8 (p<0.05) pmol/l in PD, MS, TBM and AM patients respectively whereas the level of DOPAC remained unchanged in CVD patients.

Fig. 3 shows the CSF level of serotonin (5-HT) and compared to HS, the level was significantly decreased from 8.3±1.3 pmol/l to 6.5±0.9 (p<0.05) and 5.4±0.5 (p<0.01) pmol/l in PD and MS patients respectively. The increased levels of 10.7±1.2 (p<0.01), 12.3±1.3 (p<0.001) and 10.9±0.9 (p<0.01) were found in CVD, TBM and AM patients respectively. Fig. 4 shows the CSF levels of tryptophan (TRP) and its metabolite 5-hydroxyindole acetic acid (5-HIAA). The level of TRP was significantly increased from 2.1±0.2 μmol/l to 2.7±0.3 (p<0.05), 2.95±0.2 (p<0.01) and 2.67±0.2 (p<0.05) μmol/l in CVD, MS, and AM patients, and decreased to 1.8±0.1 (p<0.05) in PD patients, and remained unchanged in TBM patients.
The level of 5-HIAA was significantly decreased from $0.46\pm0.06 \mu\text{mol/l}$ to $0.399\pm0.05 \mu\text{mol/l}$ (p<0.05) in PD patients and increased to $0.63\pm0.08 \mu\text{mol/l}$ (p<0.05) in CVD patients. The level of 5-HIAA remained unchanged in MS, TBM and AM patients.

### Discussion

DA and NA are synthesized from the amino acid tyrosine derived from food intake or protein breakdown. The main metabolites of DA are homovanillic acid (HVA) and 3,4-dihydroxyphenylacetic acid (DOPAC) and of NA, 3-methoxy-4-hydroxyphenylglycol (MHPG). The highest concentrations of DA are observed in basal ganglia and of NA in the hypothalamus. The major inactivation of monoamines is due to an active reuptake of the parent substances from the synaptic cleft into the presynaptic bouton. Apart from this reuptake, neurotransmitters and their metabolites in the synaptic cleft are cleared to the cerebrovenous blood. However, a small fraction diffuses into the CSF. Our results show that the CSF levels of NA and its metabolite MHPG are increased in all patients with neurologic disorders (Fig. 1) whereas DA is decreased in patients with PD, MS and TBM and unchanged level were found in CVD and AM patients (Fig. 2). Its metabolites, HVA and DOPAC were decreased in all patients except CVD (Fig. 2).

The elevated CSF level of NA and its metabolite MHPG in these patients indicates that the enzymes (monoamine oxidase and catechol-O-methyl transferase) converting NA into MHPG are activated under these conditions. The role of CNS and influence of various neurotransmitters on the immune systems is well documented. The CNS activity could also be influenced by immune system. These findings suggest the importance of neurotransmitters in MS and PD patients since MS is an immune modulated disease whereas PD is a disease which clearly shows DA deficiency in the brain.

Apart from catecholamines, serotonin (5-HT) plays an important role in CNS, is synthesized from the amino acid tryptophan (TRP) and is metabolized mainly to 5-hydroxyindoleacetic acid (5-HIAA). 5-HT neurons originate from the raphe nuclei in the brain stem and adjacent nuclear groups and, as the noradrenergic system, project widely to most areas in the brain. The highest concentration of 5-HT is found in subcortical nuclei. 5-HIAA and 5-HT are known to pass through the BBB but the rate of TRP entry into brain is dependent on its availability in the blood and its relationship with other large neutral amino acids through the transport system.

As previously reported, 5-HT is decreased in MS, PD patients whereas it is increased in CVD, TBM and AM patients (Fig. 3) suggesting that under different neurologic disorders the enzymes (tryptophan hydroxylase and aromatic aminooacid decarboxylase) converting TRP into 5-HT are affected, however, the enzyme (aldehyde dehydrogenase) converting 5-HT into 5-HIAA is not affected since the level of 5-HIAA was unchanged under all these neurological conditions except in PD (Fig. 4).

The possibility that alteration of neurotransmitter production and turnover may play a significant role for either the CNS or systemic changes during these diseases has received little attention. Only one study in infant rats with menigitis documented an increase in forebrain NA and DA levels at the time of acute infection and evidence of persistent perturbation of monoamines neuronal transmission in adult rats surviving the disease. These authors hypothesized that the neurotransmitters changes may explain certain neurological sequelae, such as motor hyperactivity that are observed following menigitis. While much progress has already been made in documenting biochemical alterations in various neurodegenerative disorders, considerable research remains to be done in order to establish the specificity of these findings. Preclinical studies in animal models will undoubtedly help us in understanding the interrelationship between different neurotransmitters in the brain as well as their distributions and functions. On the basis of future research on degenerative disorders, the role of various neurotransmitters, and the role of the enzymes involved in their metabolites could be clearly defined in order to
develop drug therapy with efficiency.

References


